PATENT COOPERATION TREATY

TRANSLATION

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	See form PCT/ISA/210
Applicant's or agent's file reference GP10003PC00	FOR FURTHER ACTION See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2005/003403	31.03.2005	31.03.2004

International Patent Classification (IPC) or both national classification and IPC
C12N5/06

Applicant
SCHWARTZ-ALBIEZ, Reinhard

1. This opinion contains indications relating to the following items:

- ☒ Box No. I — Basis of the opinion
- ☐ Box No. II — Priority
- ☐ Box No. III — Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV — Lack of unity of invention
- ☒ Box No. V — Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI — Certain documents cited
- ☐ Box No. VII — Certain defects in the international application
- ☒ Box No. VIII — Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/EP	Authorized officer
Facsimile No.	Telephone No.

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/EP2005/003403

Box No. I Basis of this opinion

1. With regard to the language, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This opinion has been established on the basis of a translation from the original language into the following language _____, which is the language of a translation furnished for the purposes of international search (under Rule 12.3 and 23.1(b)).

2. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

a. type of material

☐ a sequence listing

☐ table(s) related to the sequence listing

b. format of material

☐ in written format

☐ in computer readable form

c. time of filing/furnishing

☐ contained in the international application as filed.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

1. Reference is made to the following documents:

D1: K. Theunissen et al.: "Long-term Umbilical cord blood cells are preserved after ex-vivo culture in stroma-free culture" published in Chapter 14 Cord blood of the Autologous Blood and Marrow Transplantation X: Proceedings of the Tenth International Symposium, edited by K.A. Dicke and A. Keating May 2001.

D2: GUPTA PANKAJ ET AL: "Human LTC-IC can be maintained for at least 5 weeks in vitro when interleukin-3 and a single chemokine are combined with O-sulfated heparan sulfates: Requirement for optimal binding interactions of heparan sulfate with early-acting cytokines and matrix proteins" BLOOD, vol. 95, no 1, 1 January 2000 (2000-01-01), pages 147-155, XP002301975 ISSN: 0006-4971

D3: GUPTA PANKAJ ET AL: "Structurally specific heparan sulfates support primitive human hematopoiesis by

Box No. I Basis of this opinion

formation of a multimolecular stem cell niche" BLOOD, vol. 92, no 12, 15 December 1998 (1998-12-15), pages 4641-4651, XP002301976 ISSN: 0006-4971

D4: LEWIS IAN D ET AL: "Umbilical cord blood cells capable of engrafting in primary, secondary, and tertiary xenogeneic hosts are preserved after ex vivo culture in a noncontact system" BLOOD, vol. 97, no. 11, 1 June 2001 (2001-06-01), pages 3441-3449, XP002301977 ISSN: 0006-4971

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/EP2005/003403

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	6	YES
	Claims	1-5, 7-14	NO
Inventive step (IS)	Claims		YES
	Claims	6	NO
Industrial applicability (IA)	Claims	1-11	YES
	Claims		NO

2. Citations and explanations:

2. Novelty (PCT Article 33(2))

2.1 The present application does not meet the requirements of PCT Article 33(1) because the subject matter of claims 11 and 12 is not novel within the meaning of PCT Article 33(2).

D3 (Gupta et al.) discloses that different heparan sulfate molecules, which are obtained from two cell lines, have different effects on the primitive human hematopoietic development. Obtaining long-term-culture-inducing cells which must contain stem cells (LTC-IC) is promoted by addition of proteoglycans (see abstract lines 6-17). For example, heparan sulfate from bovine kidneys has a high degree of 6-O sulfate on the first N-acetylglucosamine (see page 4642, column 1, lines 31-35). The use of such a heparan sulfate (HS) promotes obtaining LTC-IC. It is also disclosed that supporting HSs are larger and are more frequently 6-O-sulfated than nonsupporting HSs (see page 4644, column 2, line 1 and lines 43-47). The sulfation pattern of HS and its effect on supporting the obtaining of LTC-IC was analyzed. The combination of cytokines with N-sulfated, N-reacetylated heparins which retain 2- and 6-O-sulfates (O-sulfated heparin) and unfractionated HSs obtained from the supernatant of supporting cells likewise obtain LTC-IC (see page 4645, column 2, last sentence). As disclosed in the application, and known from the prior art, sulfated iduronate, bis-sulfated glucosamine, glucuronate, sulfated N-acetylglucosamine, *inter alia*, are components of HS (see page 5, line 25).

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The culture medium characterized in claim 11 is therefore not novel in the light of D3.

The use characterized in claim 12 of HS for obtaining regio-modified glycans for the expansion of post-embryonal stem and progenitor cells does not differ from the use of the described HS, which is disclosed in D3. This use must therefore also lead to the expansion of the post-embryonal stem and progenitor cells. The use is therefore not novel either.

2.2 The present application does not meet the requirements of PCT Article 33(1) because the subject matter of claims 11 and 12 is not novel within the meaning of PCT Article 33(2).

2.3 Claim 9 relates to a composition which can be used for therapeutical purposes. However, a product is not novel only because it has been prepared by a novel process. A claim which characterizes a product by a preparation process is to be regarded as being directed at the product as such. Stem and progenitor cells which have been obtained by alternative processes and which permit a use in therapy are novelty-injurious (see, for example, D4, page 3447, column 1, lines 16-20). This also applies to claim 10.

2.4 The present application does not meet the requirements of PCT Article 33(1) because the subject matter of claim 1-5 is not novel within the meaning of PCT Article 33(2).

D4 (Lewis et al.) discloses *ex-vivo* stroma or stroma-free cultures for the long-term maintenance of hematopoietic cells capable of being transplanted. SCID repopulating cells, which must also contain stem cells, have not lost their multilineal differentation potential and their capacity of self-renewal and are, *inter alia*, also obtained and multiplied in MV8 stroma-free medium (see abstract, column 1, last line, to column 2, line 3 and column 3, last sentence, page 3441 column 2, lines 14-17, page 3442, column 1, last paragraph, page 3446 column 2 last paragraph, page 3447 column 1 lines 16-20 and lines 26-

28). It is emphasized that the MV8 stroma-free medium contains N-desulfated O-sulfated heparin, which is characterized in D3 (see also D2 page 148, column 2, lines 7-8). The multiplication process in this document can therefore not be distinguished from the process in claim 1. D4 must therefore be considered as novelty-injurious.

The specific culture of starting cells in "stroma-noncontact", as described in D4, must also be regarded as culturing in a stroma-free medium. These "stroma-noncontact" cultures permit an expansion of 76 or 37 times after 14 days (see abstract, last sentence, page 3444, column 1, last paragraph, table 2). The multiplied stem cells can also differentiate into myeloic and lymphatic cells (see D4, page 3446, column 2, last line - page 3447 column 1 line 6). Claim 1 is therefore not novel.

The features mentioned in dependent claims 2-5 are parameters which have not been measured in the prior art. Since the effect is identical, it must be assumed that the claimed medium was used. Claims 2-5 are therefore not novel (see PCT Guidelines 12.04).

2.5 D1 discloses the long-term transplantation of umbilical cord blood cells after *ex vivo* culture in stroma-free medium (see title). It was demonstrated that the addition of O-sulfated heparin to stroma-free medium brings about the obtaining/multiplication of LTC-ICs and NK-ICs (see page 601 lines 2-4). In the light of this information, an artificial stroma-free medium was reconstituted which permits the expansion of LTC-ICs and NK-ICs from umbilical cord blood cells (see page 601 paragraph 2). D1 therefore destroys the novelty of claims 1-5, 7-14.

3. Inventive step (PCT Article 33(3))

3.1 The present application does not meet the requirements of PCT Article 33(1) because the subject matter of claims 6-8 does not involve an inventive step within the meaning of PCT Article 33(3).

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Document D4 is considered to be the closest prior art in relation to the subject matter of claim 6. It discloses *ex-vivo* stroma or stroma-free cultures for the long-term maintenance of hematopoietic cells which are capable of being transplanted (see item 2.4). The subject matter of claim 6 therefore differs from the known process by the fact that the process is monitored by an ML-IC assay. The problem addressed by the present invention can therefore be considered that of intending to test the generated cells for their differentiation capability.

The solution proposed in claim 6 of the present application cannot be considered as being inventive for the following reasons (PCT Article 33(3)):

Such ML-IC assays are known from the prior art and have also been used for these purposes.

The same reasoning also applies analogously to dependent claims 7, 8.

The subject matter of claims 6-8 therefore does not involve an inventive step (PCT Article 33(3)).

3.2 Although claims 13 and 14 are unclear, at least the known process in claim 1 in the prior art has the ultimate aim of generating stem cells which can be used in therapy. The corresponding stem cells are of interest because they can be employed for example for the treatment of leukemias.

The subject matter of claims 13 and 14 therefore does not involve an inventive step (PCT Article 33(3)).

4. Industrial applicability (PCT Article 33(4))

4.1 Claims 12-14 relate to subject matter which, in the opinion of this Authority, falls under PCT Rule 67.1(iv). Consequently, no expert opinion has been established in respect of the industrial applicability of the subject matter of said claims (PCT Article 34(4)(a)(i)).

The PCT Contracting States do not have uniform criteria for assessing the industrial applicability of claims 12-14 in their present form. Patentability may also depend on the wording of

the claims. The EPO, for example, does not recognize the industrial applicability of claims to the medical use of a compound medical application; it may, however, allow claims to the first of a known compound or to the use of such a compound in the manufacture of a drug for a new medical application.

5. Clarity (PCT Article 6)

Claim 13 is unclear. The claim characterizes a process for the preparation of a therapeutic, but is dependent on claims 1 to 10, which relate to a process for obtaining and expanding post-embryonal hematopoietic stem cells and a composition. It is now unclear what sort of therapeutic is to be prepared. It is unclear which steps are required for this purpose. Claim 14 is dependent on the unclear claim. The same lack of clarity as for claim 13 therefore applies to claim 14. Moreover, it is unclear whether the process is a process for the preparation of a therapeutic which is suitable for the treatment of diseases or whether it is a process for the preparation of the therapeutic and the further treatment of diseases.

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/EP2005/003403

Box No. VIII **Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made: